Vol. XIX. *August, 1910.* *No. 3.*

BIOLOGICAL BULLETIN

MANGANESE OF THE LAMELLIBRANCHS.

H. C. BRADLEY,

DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF WISCONSIN, MADISON, WIS.

In 1892 Griffiths published an account of the finding of manganese in the blood of the lamellibranch, *Pinna squamosa.*[1] So far as I am aware this result has never been confirmed nor until recently has an examination of other molluscs led to an extension of this isolated fact. To the student of comparative physiology such a finding must be of considerable interest, adding one more respiratory mechanism to the list of five or six with which we are familiar. At the same time it is highly improbable that *Pinna squamosa* is the only mollusc utilizing manganese in a respiratory compound; we should expect to find the element in other forms more or less closely related to it. It is a matter of common observation that the respiratory proteins of the more highly organized animals fall into a few general types, such as hæmoglobin or hæmocyanin, and that while individual bloods may show subtle biological differences within one of these groups, there is never any difficulty in determining chemically whether a blood pigment is a hæmocyanin, a hæmoglobin, or some other typical complex. The effective respiratory mechanisms are thus quite limited, so that we do not expect to find a single member of a family possessed of a blood protein totally unlike the other members of that family.

For this reason we have extended the investigation of this point somewhat with a view to determining what other lamellibranchs are provided with a respiratory mechanism similar to that of *Pinna squamosa.* The most notable group which we

[1] Griffiths, *Compt. rend. de l'Acad. des Sci.*, CXIV., p. 840.

have thus far found to utilize the element manganese is the Unionidæ, the common fresh-water mussels. Since 1906 when the element was first noticed in the specimens obtained from the Madison Lakes of Wisconsin,[1] we have examined many hundreds of specimens from the Mississippi basin, St. Lawrence and Atlantic coast drainage. In not a single specimen has the element been wanting or small in amount. It is obvious that a single individual which failed to show manganese, or contained only a trace of it would be sufficient to cast grave doubts upon the normality of the element and lead one to ascribe an adventitious character to it. But in every case manganese has been abundant. The reactions for its identification are fortunately brilliant and decisive and at the same time indicate very well the relative amount of the element. The quantitative determinations show that the metal occurs in quite uniform amounts in the various specimens examined.

To summarize briefly the results: Some twenty-four analyses were made quantitatively upon material from about Madison. Some of these analyses were made upon single specimens of *Anadonta* or *Unio*, more were made upon a sample taken from the dried and pulverized tissues from a large number of specimens secured at one time from a given locality. Many of these analyses therefore represent the average of fifty or a hundred individuals. The average of the twenty-four analyses shows 21.8 per cent. of ash in the tissues, 4.52 per cent. manganese present in the ash and 0.95 per cent. manganese in the tissues. Mussels from the Wisconsin River averaged about 14.5 per cent. ash, 2.4 per cent. manganese in the ash and 0.35 per cent. in the tissues. From the Temagami Reserve of Ontario mussels averaged 15.4 per cent. ash, 3.1 per cent. manganese in the ash and 0.45 per cent. in the tissues. Specimens obtained from a great number of localities in Michigan, Illinois, Wisconsin, Indiana and Iowa average about 17.0 per cent. ash, 3.4 per cent. manganese in the ash and 0.60 per cent. in the dry tissue. A number of normal, average sized specimens from Lake Mendota were dissected into their more prominent tissues or organs. Analyses of these fractions gave the following results:

[1] Bradley, *Jr. Biological Chem.*, III., 151, 1907.

Tissue.	Per Cent. Ash.	Per Cent. Mn in Ash.	Per Cent. Mn in Tissue.
Muscle	6.0	4.87	0.293
Stomach; fibrous part	14.5	5.73	0.831
Stomach; non-fibrous part	32.0	4.66	1.492
Nephridial organs	27.0	5.31	1.434
Gills	33.5	4.89	1.638
Mantle	48.0	5.12	2.457
Liver	39.0	5.85	2.107
Eggs	37.0	2.024	0.749

Perhaps the most interesting result of these analyses was the presence of the element in the eggs and embryos, showing clearly that manganese is not an adventitious element picked up by the adult and held in the tissues from inability to excrete it—as, for example, iron compounds may be in the mammalian spleen after certain diseases involving great destruction of red corpuscles.

Another interesting point brought out in the above table is the exceedingly high mineral content of such a tissue as the mantle. Its ash content of 48.0 per cent. puts it in a class with mammaliam osseous tissue, though unlike the latter the mantle is soft and pliable. This is a type of tissue resembling no vertebrate organ or tissue of which we are aware. It seems probable that its function as a gland secreting the shell must have some connection with the high mineral content.

Having established as we believe the normality of the element manganese in the tissues of the Unionidæ, the question as to its origin naturally presents itself. It is hardly to be supposed that an animal of so great complexity as a lamellibranch would actually concentrate the element from its highly dilute solutions in lakes and streams. Such concentration is usually performed by lower forms of life which are then obtained as food. In the case of the Unionidæ the food origin of manganese is much more clearly apparent than is the food origin of copper in many of the hæmocyanin-bearing animals. The waters in which the mussels are found have invariably contained the brown masses of the manganiferous crenothrix mixed with diatoms, and other plancton forms which very probably contain manganese also. And this brown slime seems to be the normal food of these mussels so far as our observations extend. In Ontario

there are many lakes set in clean rocky basins, and fed by streams which leave little or no manganese stain on the rocks, and which appear to be free from the masses of crenothrix. In such lakes we have been unable to find mussels. In other lakes in the same region where seepage through glacial drift was apparent, or where the tributary streams flowed through such drift, discoloration of the stones, evidence of the presence of crenothrix, and the presence of the mussels seemed always to go together. For example in the Temagami Reserve, Lake Temagami itself is characterized by its clear water, and its clean rock basin. In certain parts occur limited areas of drift—sand and gravel—which are of insignificant amount. But though the bottom afforded, where the lake washed such drift areas, looked promising no mussels were found and the sandy stretches were apparently free from crenothrix. To the north of Lake Temagami are several lakes which lie in basins of glacial drift. In Sucker Gut, for example, sand and gravel beaches are abundant, the tributary stream flows through many miles of drift and carries enough manganese and iron in solution to stain its stones and pebbles strongly brown and black. The sand itself is stained with iron, and the brown slimy masses of crenothrix are abundant. In this lake and its tributary stream we found enormous numbers of small mussels wedged in thickly between pebbles or projecting from the sandy bottom. The many obvious examples in this region of the simultaneous presence of manganese, crenothrix and mussels, or of the absence of all three is probably more than a mere coincidence. We believe that more careful examination would show that the mussels require such manganiferous food as crenothrix and that they cannot live in waters where such food does not thrive.

In growing the mussels in aquaria the specimens always carry enough of the manganiferous organisms clinging to them so that in a few days an abundant development of the bacteria results. In this way several hundred grams of the dry organisms have been obtained for analysis. Such specimens are mixtures of a great variety of organisms and thus show large differences in chemical content. The ash content of such plancton crops vary from 24 to 76 per cent. of the dry weight; the manganese

from 0.13 to 1.84 per cent. of the dry weight. It has thus been possible to obtain, through the agency of these organisms, several grams of manganese from running water which contained about 0.0000066 per cent. of that element. The concentrating efficiency of these lower forms is therefore of a high order.

In secreting the shell, the Unionidæ deposit salts of manganese as well as of calcium and magnesium. The nacer of the shells, carefully freed from contaminating material, always gives a strong reaction for manganese; its presence in the shell is as characteristic as its presence in any of the tissues. It was thought therefore that an examination of fossil shells of the Unionidæ would be of interest in determining whether the manganese is of comparatively recent occurrence in these animals or whether it is a metabolic characteristic of long standing. So far we have had the opportunity to examine but one well preserved fossil shell. This was a specimen obtained through the courtesy of Dr. George Wagner who published a description of it in *Nautilus*, Vol. 18. The nacer of this shell was perfectly preserved, retaining its luster, though friable and crumbling to a powder easily. The fossil nacer gave 0.085 per cent. of manganese, while fresh shells of the present period frequently contain as much as 0.148 per cent. Thus it can be definitely asserted that the Unionidæ in pre-Pliocene times were using the element manganese as we find them today. It seems probable that the marine ancestors of the Unionidæ were themselves manganiferous. The fact that at least one marine lamellibranch is known makes such an assumption the more plausible.

To determine whether other marine lamellibranchs utilize manganese in this same way, an examination of the common forms along the coast of southern Massachusetts was made at the Woods Hole laboratory. In several forms the elements could usually be detected as a trace, but in such cases no import can be attached to its presence except as indicating that there is some marine low form of life serving as food for lamellibranchs, which also carries manganese. In *Pecten* the manganese was variable, sometimes large in amount, at others very small. It was frequently found abundant in the stomach contents. In *Modiola modiolus* the element was present in every specimen

examined, and it seemed to be rather uniform in amount. It was present in every tissue, and in the nephridial organs it was really abundant. In most of the tissues it was not at all comparable to the amount present in the Unionidæ, approximately 0.1 per cent. or less of the dry material. It will be remembered that the nephridial organs of *Modiola modiolus* are usually pigmented a dark brown—in all of some fifty specimens examined by us this brown pigmentation was prominent. It is possible that this lamellibranch deposits manganese obtained with its food in the nephridia in an attempt to excrete the element; that it is in this case adventitious and analogous to deposits of iron-containing pigment in mammalian tissues as the result of pathological conditions. It is interesting to note however that this marine mussel which stands morphologically fairly close to the Unionidæ, should appear to utilize the element so characteristic of the latter family.

It is our belief that other lamellibranchs will be found which, like *Pinna squamosa*, the Unionidæ and perhaps *Modiola modiolus*, utilize the element manganese in their metabolic processes. Such a chemical relationship may be useful in suggesting the lines of the evolutionary process which has led to the development of the present forms. It is our expectation to continue this line of investigation as opportunity permits.